Mikio AOYAMA* & Ryuso TANAKA**: Notable chromosome numbers in *Cymbidium lancifolium*, *C. javanicum* and *C. nipponicum*

青山幹男*・田中隆荘**: ナギラン，アキザキナギラン，マヤランの特異な染色体数

Cymbidium (Orchidaceae) includes approximately 50 species. Chromosome numbers of most of those species have been reported: 2n=40 (Tanaka & Kamemoto 1984) except for 2n=38 in *C. lancifolium* (Tanaka 1965, Mehra & Sehgal 1978) and n=19 in *C. macrorhizon* (Mehra & Sehgal 1978). The somatic chromosome numbers and the morphological features of the chromosomes of *C. lancifolium* and its allied two species collected in Japan and its neighbouring countries are reported in this paper.

Materials and methods Somatic cells in root tips of *C. lancifolium* and *C. javanicum* and those in rhizomes of saprophytic *C. nipponicum* were used for cytological investigation. Taxonomical treatment of these three species followed Maekawa (1958). But, Seth and Cribb (1984) treated that *C. javanicum* and *C. nipponicum* were synonyms for *C. lancifolium* and *C. macrorhizon*, respectively. The plant materials used were collected in natural forests shown in Tab. 1. Chromosomes were observed by the orcein staining method previously reported by Tanaka (1959) with a slight modification: actively growing root tips and rhizome tips were cut into small pieces of about 2 mm in length and were immersed in 0.002 M 8-hydroxyquinoline for five hours at 18°C. Then, they were fixed in 45% acetic acid for about 10 minutes at 5°C, and were macerated in the mixture of 45% acetic acid and 1N HCl (1:2) for 30 seconds at 60°C, before they were stained and squashed in 1% aceto-orcein.

Results and discussion The results of chromosome counts were shown in Tab. 1. Twenty plants of *C. lancifolium* from 11 localities (10 in Japan and one in China), had 2n=38 chromosomes (Fig. 1A) except one plant from Kouzushima Is., Tokyo Pref., Japan, had 2n=39 chromosomes. Thus, this species

* The Hiroshima Botanical Garden, Itsukaichi-cho, Saeki-ku, Hiroshima 731-51. 広島市植物公園.
** Botanical Institute, Faculty of Science, Hiroshima University, Naka-ku, Hiroshima 730. 広島大学理学部植物学教室.

Tab. 1. Locality, number of plants and chromosome number in the three species of *Cymbidium* investigated.

Species	Locality	No. of pls.	Chr. no. (2n)
C. lancifolium	Kouzushima Is., Tokyo Pref., Japan	1	39
	Dougashima-cho, Shizuoka Pref., Japan	1	38
	Ipponmatsu-cho, Ehime Pref., Japan	1	38
	Mugi-cho, Tokushima Pref., Japan	1	38
	Tsushima Is., Nagasaki Pref., Japan	1	38
	Minaminaka-gun, Miyazaki Pref., Japan	1	38
	Izumi-shi, Kagoshima Pref., Japan	1	38
	Hetsuka-cho, Kagoshima Pref., Japan	5	38
	Yakushima Is., Kagoshima Pref., Japan	4	38
	Amami-Oshima Is., Kagoshima Pref., Japan	2	38
	Taiwan, China	2	38
C. javanicum	Tsushima Is., Nagasaki Pref., Japan	1	43
	Yakushima Is., Kagoshima Pref., Japan	1	38
		1	57
	Taiwan, China	3	38
	Himalayas, India	2	38
	Sabah, Malaysia	1	38
C. nipponicum	Mugi-cho, Tokushima Pref., Japan	2	38

seemed to show 2n=38 commonly throughout its widely distributed areas. In all of the plants containing 2n=38 the chromosome set at mitotic metaphase showed a degradation of chromosome length from the longest (4 μm) to the shortest chromosomes (1.5 μm). Most of the chromosomes had centromeres in their median or submedian regions, except two chromosomes which had centromeres in their subterminal regions. The plant containing 2n=39 chromosomes from Kouzushima Is. showed no distinct chromosomes at mitotic prophase and metaphase. No heteropycnotic prochromosome at resting stage was observed in this plant. According to morphology and staining features the extra one chromosome seemed to be supernumeral: 2n=39=38+1 supernumeral. The chromosomes of each resting cell formed several small chromocentral blocks, chromatin threads and chromomeric granules scattered in nuclear space. Thus, morphology of the chromosomes at resting stage was categorized as the simple

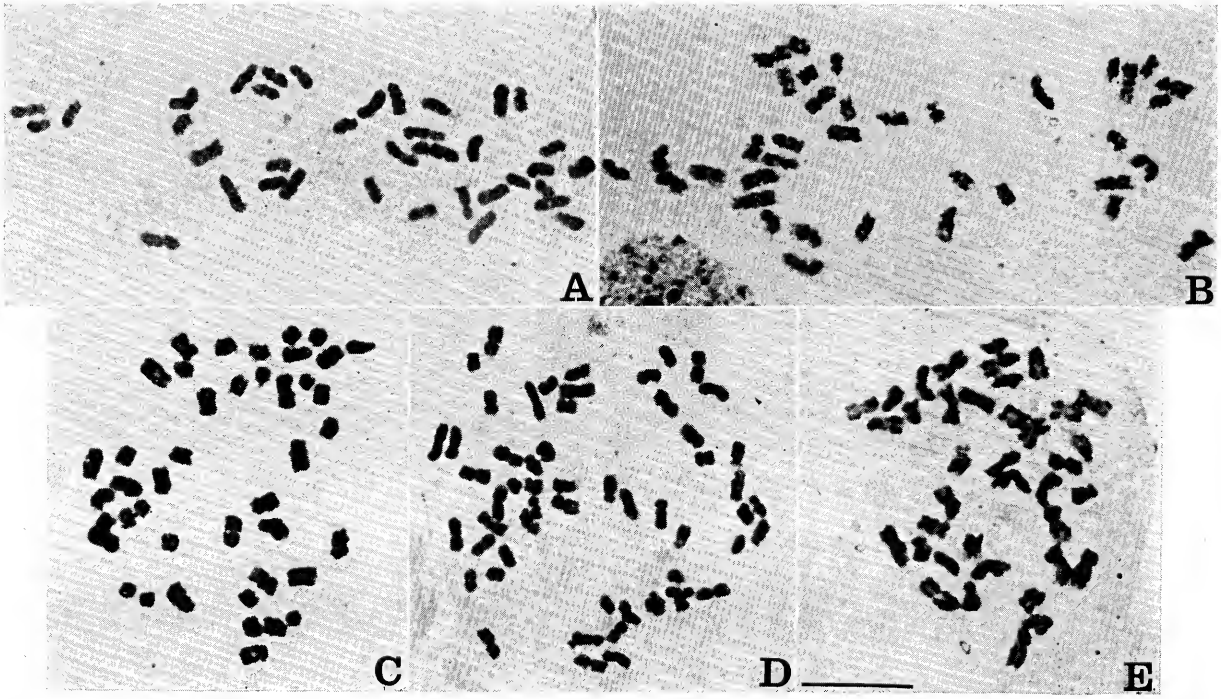

Fig. 1. Photomicrographs of somatic chromosomes at mitotic metaphase. A. *Cymbidium lancifolium* from Taiwan, 2n=38. B-D. *C. javanicum* (B. from Taiwan, 2n=38. C. from Tsushima Is., 2n=43. D. from Yakushima Is., 2n=57). E. *C. nipponicum* from Mugi-cho, 2n=38. Bar indicates 10 μm.

chromocenter type defined by Tanaka (1971). It was clearly different in resting chromosome morphology from other *Cymbidium* species containing 2n=40 chromosomes which formed many large chromocentral blocks as the complex chromocenter type by Tanaka (1971).

Among nine plants of *C. javanicum*, seven from four localities had 2n=38 chromosomes, while one plant from Tsushima Is., Nagasaki Pref., Japan, had 2n=43 chromosomes and the other plant from Yakushima Is., Kagoshima Pref., Japan, had 2n=57 chromosomes (Fig. 1B-D). The 2n=38 chromosomes at resting stage and mitotic metaphase of *C. javanicum* were similar to those of *C. lancifolium*. Among the 2n=43 chromosomes of the plant from Tsushima Is. five small chromosomes formed darkly stained chromocentral blocks at resting stage, and condensed earlier than the other chromosomes at mitotic prophase. The staining features suggested that the five small chromosomes were regarded as B-chromosomes and thus the 2n chromosome number as 2n=43=38+5B. Since among the 2n=57 chromosomes in the plant from Yakushima Is., three each of the medium and small-sized chromosomes characterized with subterminal or submedian centromeres were found, the plant was considered to be triploid with the basic chromosome number of x=19.

Two plants of *C. nipponicum* from Mugi-cho, Tokushima Pref., Japan, had 2n=38 chromosomes. Morphology of the chromosomes was found to be similar to that in *C. lancifolium* in shape at resting stage and in chromosome length and in the position of centromere.

These results and the previous report by Mehra & Sehgal (1978), suggest that *C. lancifolium* with 2n=38 seems to occur widely in Asia. In addition, it was found that *C. lancifolium*, *C. javanicum* and *C. nipponicum* also showed the same karyotypical features in chromosome number, condensation at resting stage, size of chromosomes and position of centromere at mitotic metaphase. Many experiments by various orchid breeders showed that most of the species of *Cymbidium* can be easily crossed interspecifically (cf. Sander's lists 1987). Furthermore, the cytological investigations and the hybridization data suggested that all of the interspecific hybrids were readily made in combinations among the species with 2n=40 but were not made in combinations between the species with 2n=40 and those with 2n=38. Thus, the occurence of the chromosome number of 2n=38 in *Cymbidium* could be a key to detect and clarify genetic and cytotaxonomic relationships in the species of *Cymbidium*.

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ナギランとその類縁種のアキザキナギラン，マヤランについて，日本の11場所，外国の4場所産の野生株の染色体数を算定し，3種がいずれも 2n=38 であることがわかった。但し，ナギランで 2n=38+1 過剰染色体，アキザキナギランで 2n=38+5B の各1株，アキザキナギランで 2n=57 (3倍体) 1株が見出だされた。この結果，*Cymbidium* 属には，その大多数の種が 2n=40 であるのに対して，2n=38 の小種群が存在することがわかった。

□小林義雄・大谷吉雄・萩原博光(編)：**南方熊楠菌誌** 第一巻 177 pp. 1987. 自費出版(和歌山県田辺市中屋敷町36 南方文枝)．¥7500 (送料 ¥400)．熊楠の採集した真菌類で少くとも標本，彩色図，記載のいずれかが保存されているものは4,750件ある由である。今回は1）盤菌綱102点について可能なかぎりの同定がなされ(日本新産2種)，熊楠のメモ（記載または採集データ）が活字にされている。2）膠質菌類，腹菌類，キシラリヤ，ツラフ，その他として，同定された69種のメモが活字にされ，熊楠全集から16の文章が転載されている。3）変形菌類については熊楠と弟子達によるリストを中心に，全集からの転載文がちりばめられている。近く第二巻が予定されている。県外の方は小林氏（千葉県船橋市丸山町 5-33-1）に連絡するとよい。 (三浦宏一郎)